WO 2005/003337 PCT/DK2004/000468

## **CLAIMS**

5

10

15

20

30

1. A method of producing a variant polypeptide, which method comprises:

- a) providing an amino acid sequence and a three-dimensional model for a cyclodextrin glucanotransferase (CGTase) and for an amino acid sequence for a maltogenic alpha-amylase wherein one or both models includes a substrate,
- b) superimposing the two three-dimensional models.
- c) selecting an amino acid residue in the CGTase which:
  - i) has a C-alpha atom located > 0.8 Å from the C-alpha atom of any amino acid residue in the maltogenic alpha-amylase and is located < 10 Å from an atom of a substrate.
  - ii) has a C-alpha atom located < 6 Å from a non-H atom of an amino acid residue of the maltogenic alpha-amylase corresponding to residue 190-194 of SEQ ID NO: 17, or
  - iii) is in a subsequence of the CGTase wherein each residue has a C-alpha atom located > 0.8 Å from the C-alpha atom of any residue in the maltogenic alpha-amylase sequence and wherein at least one CGTase residue has a C-alpha atom located <10 Å from a substrate, or is among the three amino acids adjacent to such subsequence in the amino acid sequence,
- d) modifying the CGTase sequence wherein the modification comprises substitution or deletion of the selected residue or by insertion of a residue adjacent to the selected residue, and
- e) producing the polypeptide having the resulting amino acid sequence.
- 2. The method of claim 1 wherein the substitution or insertion is made with an amino acid residue of the same type as the amino acid residue at the corresponding position in an alignment with the maltogenic alpha-amylase sequence, wherein the type is positively charged, negatively charged, hydrophilic or hydrophobic.
  - 3. The method of claim 1 or 2 wherein the modification of the amino acid sequence further comprises substitution of at least one amino acid residue in the CGTase sequence which is not selected.
  - 4. The method of claim 3 wherein the substitution is made with an amino acid residue of the same type as the amino acid residue of the maltogenic alpha-amylase sequence, wherein the type is positively charged, negatively charged, hydrophilic or hydrophobic.

WO 2005/003337 PCT/DK2004/000468

5. The method of any of claims 1-4 which further comprises preparing the variant polypeptide, letting it act on starch, and selecting a variant polypeptide having the ability to form linear oligosaccharide as an initial product.

## 6. A polypeptide which:

5

10

15

20

30

- a) has an amino acid sequence having at least 70% identity to SEQ ID NO: 6;
- b) compared to SEQ ID NO: 6 comprises at least one additional amino acid in a region corresponding to amino acids 194-198,
- c) compared to SEQ ID NO: 6 has a different amino acid or an insertion or deletion at a position corresponding to amino acid 16, 47, 85-95, 117, 139, 145, 146, 152, 153, 168, 169, 174, 184, 191, 260-269, 285, 288, 298, 314, 335, 413, 556, 602 or 677, and
- d) has the ability to form linear oligosaccharides as an initial product when acting on starch.

## 7. A polypeptide which:

- a) has an amino acid sequence having at least 70% identity to SEQ ID NO: 6;
- b) compared to SEQ ID NO: 6 comprises at least one additional amino acid in a region corresponding to amino acids 260-269,
- c) compared to SEQ ID NO: 6 has a different amino acid or an insertion or deletion at a position corresponding to amino acid 16, 47, 85-95, 117, 139, 145, 146, 152, 153, 168, 169, 174, 181, 184, 191, 194, 285, 288, 298, 314, 335, 413, 556, 602 or 677, and
- d) has the ability to form linear oligosaccharides as an initial product when acting on starch.
- 8. The polypeptide of claim 6 or 7 which compared to SEQ ID NO: 6 comprises 1-7 additional amino acids in a region corresponding to amino acids 194-198, particularly 5 amino acids, more particularly insertion of DPAGF, most particularly between amino acids corresponding to 196 and 197 of SEQ ID NO: 6.
  - 9. The polypeptide of any of claims 6-8, which has a different amino acid from SEQ ID NO: 6 at a position corresponding to 194-198, particularly F at a position corresponding to L195 of SEQ ID NO: 6, T at F196 or S at D197.
  - 10. The polypeptide of any of claims 6-9, which comprises an amino acid residue which is present at the corresponding position of SEQ ID NO: 17 or deletion of an amino acid residue

WO 2005/003337 PCT/DK2004/000468

in SEQ ID NO: 6 which is not present at the corresponding position in the amino acid sequence shown in SEQ ID NO: 17.

- 11. The polypeptide of any of claims 6-10, which has TLAGTDN at positions corresponding to 85-95 of SEQ ID NO: 6, YGDDPGTANHL at 260-268 or YGDDPGTANHLE at 260-269.
- 12. The polypeptide of any of claims 6-11 which compared to SEQ ID NO: 6 has a substitution corresponding to V16A, K47K, T117R, P139L, A145F, F146K, Y152F, G153V/G, Y168F, T169I, G174S, G181D, F184W, I191T, N194S, R285D, Q288T, T298I, D314E, T335A, R353H, W413R, G556S, Y602L, V677K.
  - 13. A polynucleotide encoding the polypeptide of any of claims 6-12...
- 14. A process for preparing a baked product which comprises adding the polypeptide of any of claims 6-12, or a polypeptide produced by the method of any of claims 1-5 to a dough and baking the dough to prepare the baked product.